AN X-RAY DOSE-ACTION CURVE FOR EYE-COLOR MUTATIONS IN MORMONIELLA

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Visible mutations have been induced in many widely diverse species of organisms by the use of x-rays and other ionizing radiations. The literature has been reviewed by Lea (1947) and by Catcheside (1948). Rate of such mutations is low, only one-tenth to one-fifteenth that of lethals, and hence conclusions in regard to effect of intensity differences, fractionation of dose, wave-length and combination with other factors such as oxygen pressure, infra-red rays and temperature have been drawn largely from work with lethals. Because, insofar as data have been accumulated, the proportion of visibles to lethals appears to be the same under different conditions of irradiation, these conclusions have seemed justified.

Dose-Action Curves

Dose-action curves for visibles have been shown in several organisms and have, within the limits of error of the experiments, proved similar to those for lethals. The curves formed are of the straight-line type of direct proportionality, meaning that, for a given increment of dose at any interval within the range, a similar proportion of mutations is added.

Drosophila. The most satisfactory information regarding dose-action curves for visibles is available from work with the fruit-fly *Drosophila melanogaster* (Timoféeff-Ressovsky and Delbrück, 1936). Two methods were used for identifying sex-linked visibles—the attached-X and the C1B. The former is the more convenient. Wild-type males are x-rayed and mated to females with their X-chromosomes attached so that the offspring are one hundred per cent non-disjunctional. Since the sons receive only paternal and therefore treated X-chromosomes, any visibles induced may be observed without further breeding. The C1B method is much more laborious. The treated wild-type males are mated to C1B females (females having in one X-chromosome an inversion preventing crossing-over, a recessive lethal factor and the factor for bar eyes) and the bar (C1B) daughters from this cross are set in individual cultures. If a visible is induced in a given X-chromosome, all the males in a culture from a bar female receiving that chromosome will show the mutant trait.

Table I shows data re-arranged from Timoféeff-Ressovsky and Delbrück (1936). Linearity is indicated by both methods, but percentages for total visibles recognized by the C1B are considerably higher for each of the three doses given. As pointed out by the authors this is not caused by any errors in dose, because the males from

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TABLE I

X-ray dose-action data re-arranged from Timoféeff-Ressovsky and Delbrück (1936) on sex-linked mutations in Drosophila melanogaster as identified by C1B and attached-X methods. The data are presented as—mutations/total cultures (or total males) = percentage (lower—upper 0.95 confidence limits)

Dose r units	C1B method. Total visibles	Attached-X method. Total visibles	Attached-X method. Seven selected
0	0/2378 = 0 (.00– .15)	0/7936 = 0 (.00–.05)	
1500	10/4583 = 0.22 (.10– .40)	13/9317 = 0.14 (.07–.24)	9/18,000 = .05 (.02–.10)
3000	16/3396 = 0.47 (.27– .77)	21/8442 = 0.25 (.15–.38)	28/22,500 = .12 (.08–.18)
6000	15/1809 = 0.83 (.46–1.37)	26/5183 = 0.50 (.33–.73)	32/14,500 = .22 (.15–.31)
	Cultures—12,166	Males—30,878	Males—55,000

the same lot were rayed at the same time for both methods. The disparity is due rather to the fact that the mutant type appears as a single individual in the attached-X method but as all the males of a culture produced by a C1B female. A single mutant may be missed by the observer, especially if the trait is not very distinct, or it may fail to develop if the mutant type is of lowered viability. It is obvious that the labor of dealing with the 12,166 C1B cultures in search of total visibles must have been far greater than that of rearing and observing the 30,878 sons from the attached-X mothers.

A method of avoiding both the viability disadvantage and the subjective factor causing failure to observe some of the less distinct visibles is to select a limited number of easily recognized traits of high viability which may then be used in an attached-X experiment. This was utilized by the authors who selected seven sex-linked mutant traits (w^e, w, y, v, m, g, f) in a test in which 55,000 sons of treated wild-type males were counted. Percentage of mutations noted is here less than half that for total visibles but undoubtedly a high degree of accuracy was attained. Dose-action linearity is again indicated.

In Table I lower and upper 0.95 confidence limits are presented as calculated from Ricker's (1937) table for Poisson frequency distributions. The authors give

TABLE II

X-ray dose-action data on eye-color mutations from Woods Hole wild-type stock of Mormionella vitripennis (Walker)

Dose r units	Mothers	Total sons	Sons per mother	Mutants					
				Bright-eyed					Dark red
				Scarlet	Orange	Oyster	Total	% (0.95 confidence limits)	
0	632	18,039	28.54	0	0	1	1	.0055 (.00055–.03104)	0
1340	729	16,011	21.96	17	2	3	22	.14 (.08–.21)	8
2680	874	10,058	11.51	17	3	5	25	.25 (.16–.37)	5
4020	853	5,268	6.18	20	2	4	26	.49 (.32–.72)	1
5360	855	2,708	3.17	17	3	3	23	.85 (.54–1.27)	0
Totals	3942	52,084		71	10	16	97		14

standard errors following percentages of mutations. Standard errors are not applicable to very low percentages because of asymmetry in the distributions. Ricker's method has likewise been followed in Table II and in Figure 1 for our Mormoniella data.

A dose-action curve for production of visibles in the X-chromosomes of the egg might be obtained by x-raying the females of Drosophila and examining their sons. Their daughters also might be set individually and the F_2 males examined. The former procedure would be less laborious but would, like the attached-X method, be less satisfactory because of the subjective factor in failure to recognize less dis-

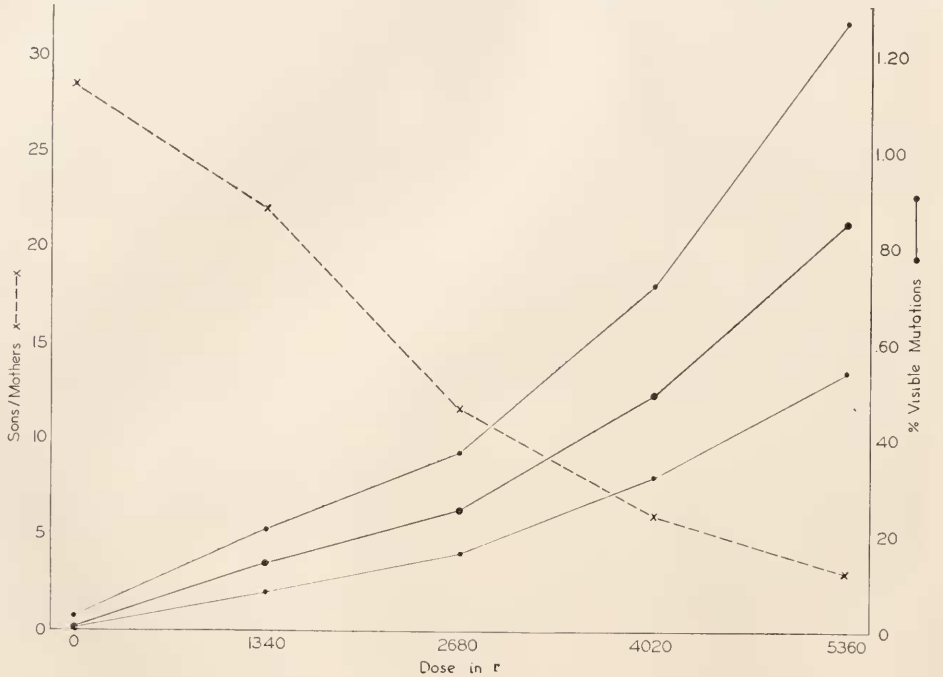

FIGURE 1. Average numbers of sons per mother and percentages of eye-color mutations produced by Mormoniella females, control and x-rayed with different doses. Lower and upper 0.95 confidence limits calculated from Ricker's (1937) table are given for the mutation percentages.

tinct types and because of reduced viability of some of the mutants. Testing of daughters, although laborious, would have the advantage, as in the C1B method, of many mutants resulting from the same mutation, but only half, instead of all, of the males in the fraternity would be expected to show the mutant trait. This last point might be advantageous in the case of less distinct mutant types because wild-type sibs with the same residual heredity as their mutant brothers would be present for comparison. Relatively little has been published with regard to radiation of females in Drosophila, aside from a few abstracts which have recently appeared. Now, however, these investigations are actively under way. Differences in dose-action relationships are to be expected, not only between sperm and egg, but between different meiotic stages of the latter.

Habrobracon. Many x-ray visibles have been produced in the parasitic wasp *Habrobracon juglandis* (Ashmead) by radiation of both males and females (Whiting, 1932, 1934, 1935). Treatment of unmated females results in mutant sons. Treatment of males gives rise to F_2 fraternities in some of which half the males are mutant. The same occurs following the mating of treated females to untreated males. When mated females are treated both eggs and sperm are exposed and mutants may appear singly as F_1 males and as many males in some F_2 fraternities. A few dominants have been produced as in Drosophila which may show directly in the heterozygous females and some recessive mutations have been recognized because of their dominance over a third allele in a compound female. While Habrobracon has the theoretical advantage of male haploidy, there are practical disadvantages (web- and cocoon-spinning of both parasite and host) making isolation of virgin females and collection of food more laborious. For these reasons dose-action curves have not as yet been obtained.

Mormoniella. In the chalcidoid wasp *Mormoniella vitripennis* (Walker) numerous x-ray-induced eye-color mutations from wild type (dark brown) have already been reported (Whiting, 1951). These range from dark red through tomato, vermilion, scarlet and peach to "oyster," the last named being devoid of pigment and transparent so that the black color of the underlying integument shows through. Thus the appearance of the eye suggests an oyster. When oyster wasps are placed in alcohol, the eyes become opaque white. The eye colors ranging from tomato to oyster may be classed as "bright." All of these that have been tested have proved to be hereditary, dependent upon recessive gene differences from wild type. The dark reds are for the most part also hereditary but occasionally one is found which has bred as a somatic overlap from wild type. Some very dark reddish-brown types occurring in wild stock have proved to be hereditary. These are readily separated as "red-eyed pupae" from their sibs which have dull reddish-brown eyes in the late pupal stage.

Spontaneous eye-color mutations are rare or at most very infrequent in pure stocks, either wild-type or mutant-type. Spontaneous "mutants" are relatively frequent among the offspring of heterozygous females. However, some at least of these "mutants" are recombinants. The problem of spontaneous mutation is being further investigated.

All of the induced eye-color mutations have been obtained by radiation of females. The majority have appeared as single sons of unmated females but in a few cases, when treated females were crossed to untreated males, the mutants from any one mutation constitute about one-half of the sons of a single daughter from the cross. Because testing daughters individually is time-consuming, rates for visible viable mutations are determined from inspection of haploid sons of treated females. By exclusion of dark reds and reddish-browns from the final calculations, the subjective factor is reduced to a minimum.

Genetic evidence indicates that all males are haploid (Whiting, 1951). Biparental diploid males such as occur as sex homozygotes in the ichneumonoid wasp Habrobracon have not been found in Mormoniella. Females carry genes derived from both parents, but sons of mated females as of unmated are gynogenetic except for rare instances interpreted as possible androgenesis. Sex determination must then be different from that in Habrobracon as it has been shown to be in the

related chalcidoid Melittobia (Schmieder and Whiting, 1947). The mechanism of sex determination has yet to be discovered for this group.

During the summer of 1952 tests were made at the Marine Biological Laboratory by David T. Ray of wild-type and of various mutant stocks of Mormoniella to determine which might be more suitable for a dose-action curve. Wild-type (WH+) was selected, a stock inbred from wasps that were infesting fly pupae at the Supply Department dock on the Eel Pond. Female pupae were isolated and freshly eclosed virgins or very dark female pupae were placed in gelatine capsules for raying. Treatments were given with the x-ray apparatus having two tubes in alternate parallel, cross-firing through the specimens which were placed 13 centimeters from the targets. KVP was 182, MA 25, equivalent filtration .152 mm. Cu, intensity approximately 2680 r/min. Rayings were made on 26 different days. Females from each host puparium were divided among the capsules given the different doses as a precaution to equalize distribution in case a mutant trait were running in the stock. Among the 52,084 sons of 3,942 mothers there were 97 mutants classed as having "bright" eyes and 14 with eyes dark red (Table II). While none of the latter occurred among the 18,039 controls, their distribution was irregular among the treated. Rate is higher but not significantly so among those from females given 1340–2680 r (13/26069), .050% (.026–.086), than among those from females given 4020–5360 r (1/7976), .013% (.001–.070). Rate for total treated (14/34045), .041% (.023–.069), is significantly higher than the zero rate for the controls (0/18039), 0.0% (0.000–0.021). Test of one dark red from the 2680 r treatment showed that he bred as wild type. The others were not tested.

The 97 bright-eyed mutants were classed as scarlet, orange and oyster. One oyster appeared among the controls. Scarlet, represented by 71 mutants among the total 97, is by far the most frequent. Only in one instance were two eye mutants found in a single vial and these were different, a scarlet and an oyster from the 5360 r treatment. Each bright-eyed mutant may then be considered to result from a separate mutation.

Stage of meiosis at time of treatment is of interest. With the controls and lower treatments mutations might appear from first meiotic metaphase as well as from prophases. With higher treatments, 2000 r and above, few if any metaphase-treated eggs would be expected to produce offspring, if inferences may be drawn from lethal rates in Habrobracon. It is very unlikely that any progeny have been included from treated gonial cells because transfers were not made to new vials. Death of offspring developing from eggs treated as young oocytes, if indeed the parents survived to lay such eggs, would be expected to result from exhaustion of food supply. Replication could occur from mutations in early gonial divisions only, since a single egg and its accompanying nurse cells comprise the products of the last four gonial divisions. Failure of similar mutant types to appear in the same vial is in agreement with this expected lack of replication. However, the presence of similar mutant types in one vial would not prove replication. They should occur rarely by chance from two separate mutations, especially in the case of scarlet, the most frequent mutant type.

Figure 1 shows percentage of bright-eyed mutants increasing with increasing dose. The curve dips at 2680 r but this dip is not a significant departure from the straight line expected on the basis of single hits producing the mutations.

A method of calculating goodness of fit of these data to a straight line has been suggested by Dr. Sewall Wright (Table III). Since doses given were simple multiples of the minimum dose, 1340 r, this may be taken as the unit dose. "Wasp-doses" are then the number of surviving wasps multiplied by the number of 1340 r units to which the eggs were subjected. Ninety-six bright-eyed mutants resulted from 62,763 "wasp-doses." Distribution of mutants calculated (c) on the basis of 1340 r having the same chance (96/62,763 = .00153) of producing a mutation, regardless of amount of dose, would then be in proportion to the distribution of "wasp-doses" among the survivors. From the differences between the observed number of mutants (o) and the calculated (c), chi square, 3.97, was obtained. This deviation is insignificant.

Average numbers of sons per mother (Table II and Figure 1) show decrease with increasing dose. Females subjected to x-radiation become sterile or die

Table III

Calculation of goodness of fit to linearity of bright-eyed Mormoniella mutants from x-rayed mothers. Data from Table II. (Method suggested by Dr. Sewall Wright)

Dose in 1340 r units	Total sons	"Wasp-doses"	Mutants		$\frac{(o-c)^2}{c}$
			(o)	(c)	
1	16,011	16,011	22	24.49	.25
2	10,058	20,116	25	30.77	1.08
3	5,268	15,804	26	24.17	.14
4	2,708	10,832	23	16.57	2.49
Totals	34,045	62,763	96	96.00	3.97

$\chi^2 = 3.97$ n = 3 P = .26

after four or five days. Many of the untreated also die at this time but a minority may be transferred to fresh host pupae and will produce further offspring. In the present experiment transfers were not made so that average potential fecundity of controls is higher than that indicated.

Discussion

Dose-action curves for visibles in Drosophila are essentially linear, indicating single hits producing the mutations. There is no dip at the mid-point (3000 r), and in two of the three experiments a slight rise occurs. The dose-action curve for bright-eye-color mutations in Mormoniella is likewise consistent with linearity but there is a dip at the mid-point (2680 r). However, the confidence limits are wide enough both in Drosophila and in Mormoniella to permit an hypothesis either of uninterrupted linearity or of a dip (at 2680–3000 r). Neutron experiments (unpublished) indicate a dip in the dose-action curve for eye colors in Mormoniella. More extensive x-ray experiments are in progress with Mormoniella which should narrow the confidence limits sufficiently to establish definitely whether or not a dip is present. Comparison may then be made with the neutron tests and the significance of the dip may be considered.

Summary

1. X-ray dose-action curves for visible mutations in Drosophila are discussed. An x-ray dose-action curve for eye-color mutations in Mormoniella is presented. Within the limits of error of the experiments the curves may be of the straight-line type indicating that single hits produce the mutations. However, in the Mormoniella curve an insignificant dip occurs at the mid-point, 2680 r, suggesting the possibility of a second factor.

2. A shortened chi square method of testing goodness of fit to a straight line is presented. With reference to the present Mormoniella data, the deviation is shown to be insignificant.

LITERATURE CITED

Catcheside, D. G., 1948. Genetic effects of radiations. *Advances in Genetics,* **2**: 271–358. Academic Press, New York.

Lea, D. E., 1947. Actions of radiations on living cells. The Macmillan Co., New York.

Ricker, Wm. E., 1937. The concept of confidence or fiducial limits applied to the Poisson frequency distribution. *J. Amer. Stat. Assoc.,* **32**: 349–356.

Schmieder, R. G., and P. W. Whiting, 1947. Reproductive economy in the chalcidoid wasp Melittobia. *Genetics,* **32**: 29–37.

Timoféeff-Ressovsky, N. W., and M. Delbrück, 1936. Strahlengenetische Versuche über sichtbare Mutationen und die Mutabilität einzelner Gene bei *Drosophila melanogaster. Zeitschr. indukt. Abstramm.-u. Vererb.,* **71**: 322–334.

Whiting, P. W., 1932. Mutants in Habrobracon. *Genetics,* **17**: 1–30.

Whiting, P. W., 1934. Mutants in Habrobracon, II. *Genetics,* **19**: 268–291.

Whiting, P. W., 1935. Recent x-ray mutations in Habrobracon. *Proc. Pa. Acad. Sci.,* **9**: 60–63.

Whiting, P. W., 1951. Multiple complementary alleles in Habrobracon and Mormoniella. *J. Genetics,* **50**: 206–214.